nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.

37. A method of determining if a seed is germinating comprising:

providing a seed suspected of germinating;

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isolating an extract from the seed; and

measuring levels of nonsymbiotic plant hemoglobin expression within the extract,

wherein high levels of nonsymbiotic plant hemoglobin expression indicate that the seed is germinating.

38. The method according to claim 37 wherein the nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.

Copies of specification pages 26-27, amended in accordance with the claim amendments described above, are enclosed, as are copies of pages 1, 26 and 27 with the changes indicated thereon.

REMARKS:

As the examiner can see, the claims have been amended to state the essential elements of applicants' invention. Support for the claim amendments may be found on, for example, page 7 of the application as filed.

Claims 28-34 were rejected under 35 USC 112. Applicants believe that the amendments to claim 28 overcome this objection.

Claim 28 was rejected under 35 USC 102 as anticipated by Jacobsen-Lyon et al. Applicant notes that the cited reference found expression of nonsymbiotic hemoglobin in some plant tissues. However, this reference does not teach improving agronomic properties of a plant by providing the plant

increased levels of nonsymbiotic hemoglobin. Rather, this reference analyzed the promoter of a nonsymbiotic hemoglobin gene using reporter gene constructs. Furthermore, applicants note that on page 219, first paragraph, last 3 lines, Jacobsen-Lyon states "until we have mutant plants available, we will not be certain of the function of plant hemoglobins in non-symbiotic tissues". Thus, Jacobsen-Lyon does not teach that there would be any benefit to increasing levels of non-symbiotic hemoglobin in a plant cell. Applicants believe that in view of the above arguments and the amendments to claim 28, this rejection is overcome.

Claims 28-38 were rejected under 35 USC 103(a) in view of Bailey and Sowa. Applicant notes that as stated in the information disclosure statement filed January, 2002, the Sowa reference appeared in the August 1998 issue of Proceedings of the National Academy of Sciences USA and is therefore not prior art, as the provisional application on which the instant application claims priority on (60/090,929) was filed on June 26, 1998, prior to the publication of this reference. As the Examiner will note, page 1, paragraph 1 has been amended to include reference to the prior applications.

The examiner's assistance and helpfulness in this matter has been greatly appreciated. Further and more favorable consideration of the application in view of the amendments is respectfully requested.

Respectfully submitted
Philip Guy et al.

PER: ______________________
MICHAEL WILLIAMS
Registration No: 45,333

MRW/dj
Enc.(2)

Michael R. Williams Winnipeg, Manitoba,, Canada
Telephone (204) 947-1429
FAX (204) 957-0516

HEMOGLOBINS TO MAINTAIN CELL ENERGY STATUS

<u>This application is a National Phase entry of PCT CA99/00587, having an international filing date of June 24, 1999 and this application claims priority under 35 USC § 119(e) to USSN 60/106,638, filed November 2, 1998 and to USS 60/090,929, filed June 26, 1998</u>

The present invention relates generally to the field of expression vectors and transgenic organisms.

BACKGROUND OF THE INVENTION

Hemoglobins are widespread throughout the biosphere (Wittenberg and Wittenberg, 1990, *Annu Rev Biophys Biophys Chem* **19**:217-241). They are found in a broad range of organisms from bacteria, through unicellular eukaryotes, to plants and animals, suggesting that they predate divergence of life into plant and animal forms. Plant hemoglobins have been classified into symbiotic and nonsymbiotic types (Appleby, 1992, *Sci Progress* **76**:365-398): symbiotic hemoglobins are found in plants that are capable of participating in microbial symbioses, where they function in regulating oxygen supply to nitrogen fixing bacteria; nonsymbiotic hemoglobins have only recently been discovered and are thought to be the evolutionary predecessors of the more specialized symbiotic leghemoglobins. The ubiquitous nature of nonsymbiotic hemoglobins is evidenced by their broad presence across the plant kingdom (Appleby, 1985, <u>Nitrogen Fixation and CO_2 Metabolism</u>, eds. Ludden and Burris, pp. 41-51) and the widespread presence and long evolutionary history of plant hemoglobins suggest a major role for them in the life of plants.

Specifically, plant hemoglobins have been known to exist in the root nodules of legumes for almost 60 years (Kubo, 1939, *Acta Phitochem* **11**:195-200; Keilen and Wang, 1945, *Nature* **155**:227-229). Over the years, hemoglobins have been positively identified in three non-leguminous dicotyledonous plants: *Parasponia andersonii*, *Tream tomentosa*, and *Casuarina glauca* (Appleby et al., 1983, *Science* **220**:951-954; Bogusz et al., 1988, *Nature* **331**:178-180; Kortt et al., 1988, *FEBS Lett* **180**:55-60). Recently, an Hb cDNA from barley was isolated and the gene was demonstrated to be expressed in seed and root tissues under anaerobic conditions (Taylor et al., 1994, *Plant Mol Biol* **24**:853-862), providing further evidence to support the contention that plant hemoglobins have a common origin (Landsmann et al., 1986, *Nature* **324**:166-168). Since Hb has now been demonstrated to occur in two of the major divisions of the plant kingdom, it is likely

<u>CLAIMS</u>

28. A method of improving the agronomic properties of a plant comprising:

<u>maintaining plant vigor and hardiness under stressful conditions by</u> providing [a] <u>the</u> plant [having] <u>with</u> increased cellular levels of a nonsymbiotic plant hemoglobin; and

growing the plant <u>under stressful conditions, thereby allowing the plant to develop more vigorously under adverse conditions</u>.

29. The method according to claim 28 wherein the nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.

30. The method according to claim 28 wherein the improved agronomic properties include germination.

31. The method according to claim 28 wherein the improved agronomic properties include seedling vigour.

32. The method according to claim 28 wherein the improved agronomic properties include reduced cellular levels of fermentation products.

33. The method according to claim 28 wherein the improved agronomic properties include increased oxygen uptake.

34. The method according to claim 28 wherein the improved agronomic properties include increased tolerance to hypoxic conditions.

35. A method of selecting seeds for breeding to produce seed lines having desirable characteristics comprising:

providing a representative seed of a given seed line;

growing the seed such that the seed germinates;

isolating an extract from the seed;

measuring levels of nonsymbiotic plant hemoglobin expression within the extract; and

selecting or rejecting the seed for further breeding based on the hemoglobin levels.

36. The method according to claim 35 wherein the nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.

37. A method of determining if a seed is germinating comprising:

providing a seed suspected of germinating;

isolating an extract from the seed; and

measuring levels of nonsymbiotic plant hemoglobin expression within the extract,

wherein high levels of nonsymbiotic plant hemoglobin expression indicate that the seed is germinating.

38. The method according to claim 37 wherein the nonsymbiotic plant hemoglobin is barley nonsymbiotic hemoglobin.